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Some New and Reconfirmed Biological Observations in Two Species of *Eugymnanthea* (Hydrozoa, Leptomedusae, Eirenidae) Associated with Bivalves

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Abstract. New observations on the morphology and the relationship with the host of two species of bivalve-inhabiting hydrozoans *Eugymnanthea* (Eirenidae) are reported. The mature medusae of Mediterranean *Eugymnanthea inquilina* and Japanese *E. japonica* were clearly distinguishable by their two diagnostic features (presence/absence of a manubrium and the number of statoliths per statocyst), and exhibited a different attachment manner of their hydroids to the gill of their common host *Mytilus galloprovincialis*. In order to determine if the differences in the diagnostic features are due to differences in salinity, *E. japonica* hydroids were raised under Mediterranean conditions (40‰), which are never experienced in Japan (30‰). However, the medusae released from these hydroids still exhibited the typical Japanese features, suggesting they may be under genetic, rather than environmental, control. Additional new observations, including the association of *E. inquilina* with two unrecorded species of bivalves, and the appearance of hermaphroditic medusae in other populations, including the one collected from the type locality, are reported.

Key words: *Eugymnanthea japonica*, *Eugymnanthea inquilina*, morphology, attachment, new host, hermaphrodite.

Introduction

Eugymnanthea inquilina Palombi and *E. japonica* Kubota, the most derived bivalve-inhabiting hydrozoans in the Eirenidae, are extremely similar in morphology. Differences are detected only in the adult medusae, and even then, there is an overlap in the range of diagnostic traits. Two differences are observed: *E. inquilina* usually, but not always, lacks a manubrium and has more than two statoliths per statocyst, while *E. japonica* usually, but not always, has a manubrium and 1–2 statoliths per statocyst. In many hydrozoans, uncertainty over whether morphological differences are genetic or environmental has resulted in difficulty in species identification. The observed differences in *Eugymnanthea* could either be genetic, possibly due to parallel evolution (Kubota, 2000), or they could be environmental. While both species occupy the same species of host bivalves, primarily *Mytilus galloprovincialis*, the external environmental conditions are different. *E. japonica* is found only in the western Pacific, where the salinity ranges from 30 to 34‰. *E. inquilina* is found only in parts of the Mediterranean, where the salinity ranges from 35 to 40‰.

The aim of this study is to determine if the diagnostic morphological features in the mature medusae of the western Pacific species, *E. japonica*, are affected by environmental conditions, particularly salinity, by culturing them in the laboratory under Mediterranean conditions. Additionally, some new biological findings on both the host bivalve species and the locality of *E. inquilina* are reported here, including reconfirmation of the manner of attachment of the hydroids to the gill of the common host, *Mytilus galloprovincialis*.

Materials and Methods

To collect *E. inquilina* hydroids, three species of bivalves (*Mytilus galloprovincialis* Lamarck, *Mytilaster minimus* (Poli), and *Chlamys glabra* (Linnaeus)) were collected from 4 localities in southern Italy (Table 1). Many mussels of *M. galloprovincialis*, with or without hydroids, were kept in the laboratory at the University of Lecce, Lecce, Italy, from mid-October, 1999 to mid-March, 2000 under constant conditions. Mussels were placed in glass or plastic containers filled with natural seawater obtained from the rocky coasts near Porto Cesareo or inside Porto Cesareo, Ionian Sea, SE Italy (salinity 38–40‰). The seawater was aerated and the cultures were kept at 23°C and 15L:9D photo period. They were fed newly hatched *Artemia* nauplii, and water was changed nearly every day at least once. Every morning, the cultures were examined under a microscope for released medusae, before they had a chance to spawn. Also, large hydroids associated with each specimen of the new host species (*Mytilaster minimus* and *Chlamys glabra*) were collected and reared in the same conditions.

Table 1. Collection data and association rate of two species of *Eugymnanthea*.

Bivalve species collected (*n*)	Collecting date	Locality	Shell size (range in mm)	Association rate (%)
E. japonica from Japan				
Mytilus galloprovincialis (27)	4-X-1999	Atami	38–51 (APA)	S
Mytilus galloprovincialis (20)	11-X-1999	Shirahama	19–27 (APA)	S
E. inquilina from Italy				
Mytilus galloprovincialis (131)	18 & 21-X-1999	Torre dell'Inserraglio	19–38 (APA)	4.6
Mytilus galloprovincialis (72)	13 & 27-X-1999, 26-XI-1999, 4-I-2000	Taranto (Mar Grande and Mar Piccolo)	17–63 (APA)	54.2
#*Mytilaster minimus* (45)	4-I-2000	Taranto (Mar Piccolo)	12–24 (APA)	2.2
#*Chlamys glabra* (73)	23-I-2000, 6-II-2000	Taranto	26–45 (shell length)	11.0
**Callista chione* (33)	16-I-2000, 26-II-2000	Taranto	42–68 (shell length)	0
Mytilus galloprovincialis (60)	24-XI-1999	Lago Fusaro	42–68 (APA)	23.3
**Tapes decussatus* (19)	24-XI-1999	Lago Fusaro	28–44 (APA)	0
**Arca noae* (63)	23-I-2000, 6-II-2000	Porto Cesareo§	32–76 (shell length)	1.6
Mytilus galloprovincialis (10)	6-II-2000	Porto Cesareo	18–50 (APA)	0
Mytilus galloprovincialis (13)	12-I-2000	Frigole	18–33 (APA)	0
Mytilus galloprovincialis (77)	10-III-2000	Torre del Serpe	23–46 (APA)	0

S: Specimens associated with hydroids were selected. APA: Antero-posterior axes. *: Collected by fishermen (bought at a seafood market). #: New host species. §: New locality.

Table 2. Number of medusae examined in two species of *Eugymnanthea*, showing new findings.

Host species	No. of hosts exam.	Locality	No. of medusae examined			
			Female	Male	Hermaphrodite	Sex undetermined (=spent)
E. japonica from Japan						
Mytilus galloprovincialis	27	Atami	367	55	0	66
Mytilus galloprovincialis	20	Shirahama	8	12	0	25
E. inquilina from Italy						
Mytilus galloprovincialis	5	Torre dell'Inserraglio	13	7	0	5
Mytilus galloprovincialis	24	Taranto	15	12	2	0
#*Mytilaster minimus*	1	Taranto	2	0	0	1
#*Chlamys glabra*	5	Taranto	4	1	2*	0
Mytilus galloprovincialis	2	Lago Fusaro	3	23	12*	7

#: New host species. *: New findings.

E. japonica specimens were obtained by collecting *M. galloprovincialis* attached to a mooring raft from two localities in Japan (Salinity *ca* 30‰), and were transported to the laboratory at the University of Lecce and reared under conditions identical to those described above for *E. inquilina*. Morphological features were examined under a microscope.

Results and Discussion

No distinct morphological changes were detected during the entire course of rearing of *E. japonica* under high salinity Mediterranean conditions (38–40‰), which are never experienced in their natural habitat in Japan (*ca* 30‰). No changes were observed in the morphology of laboratory-released medusae from the hydroids originating from La Spezia, Italy, that were kept for a month in a seawater of lower salinity in Japan (Kubota, 1989). This suggests that the morphology may be under genetic, rather than environmental, control. The two diagnostic characters, the presence or absence of the manubrium and the number of statoliths per statocyst (see Kubota, 2000), appear to be very reliable for distinguishing these two closely related species (Tables 3, 4). Note, however, that the hydroid stage of the two species is morphologically indistinguishable.

We also observed that *E. inquilina* hydroids rarely attach to the gill of *Mytilus galloprovincialis* (Table 5), as was previously observed (Kubota, 1989; Piraino *et al.*, 1994). If the hydroids are attached to the gill, the number of polyps on this site is very small, whereas many hydroid polyps are seen attached to other parts of the same host species. We speculate that the hydroids of *E. inquilina* found on the gill may have moved there from other regions of the host

Table 3. Difference of frequency in two diagnostic characters in two species of *Eugymnanthea*.

Host species	Locality	Manubrium		No. of statoliths per statocyst							
		Present	Absent	0	1	2	3	4	5	6	7
E. japonica from Japan											
Mytilus galloprovincialis	Atami	439	31	81	2371	399	42	3	1		
Mytilus galloprovincialis	Shirahama	43	2	3	227	20					
E. inquilina from Italy											
Mytilus galloprovincialis	Torre dell'Inserraglio	0	25	30	79	68	23				
Mytilus galloprovincialis	Taranto	0	29	6	53	85	37	11	5	0	1
#*Mytilaster minimus*	Taranto	0	3	0	16	7	2				
#*Chlamys glabra*	Taranto	0	7	4	25	21	5	1			
Mytilus galloprovincialis	Lago Fusaro	0	45	9	57	164	87	16	3		

#: New host species.

Table 4. Difference of frequency in the diagnostic character (Stl=number of statoliths in a medusa) in three categories (St=7, 8, 9) of two species of *Eugymnanthea*.

Host species	Locality	Number of statocysts per medusa: St=8						St=9		St=7		
		Stl:1–7	8–15	16–23	24–31	32–39	40–47	9–17	18–26	1–6	7–13	14–20
E. japonica from Japan												
Mytilus galloprovincialis	Atami	20	290	2				11		3	55	1
Mytilus galloprovincialis	Shirahama	1	21					1			4	
E. inquilina from Italy												
Mytilus galloprovincialis	Torre dell'Inserraglio		6	14	5							
Mytilus galloprovincialis	Taranto			9	6	1	1					2
#*Mytilaster minimus*	Taranto			2					1			
#*Chlamys glabra*	Taranto			2	3				1			
Mytilus galloprovincialis	Lago Fusaro		1	11	21	2			1			

#: New host species.

Table 5. Attachment of hydroids of *Eugymnanthea inquilina* to various body portions in both sides of the mantle cavity of *Mytilus galloprovincialis*.

Locality	Host size (mm)*	Gill	Visceral mass	Mantle	Labial palp
Torre dell'Inserraglio	25, 28, 32, 32	– –	– –	+ –	– –
	32	– –	+ –	+ +	+ –
	32	– –	+ +	+ +	+ +
Taranto	38, 46	– –	– –	+ –	– –
	39, 48	– –	+ +	+ –	– –
	39	+ –	+ +	+ –	– –
	41	– –	– –	+ –	– –
	42	– –	– –	+ +	– –
	48	– (+)	+ +	+ +	+ +
	49	– –	+ –	– +	+ +
	49	– –	+ +	+ –	+ +
	49	(+) (+)	+ +	+ +	+ +
	51, 54	– –	+ +	+ +	– –
	51	– –	+ +	+ +	– +
	52	– –	+ +	+ +	+ +
Lago Fusaro	44, 45, 52	– –	– –	+ –	+ –
	45	– –	– –	+ –	– –
	49	– –	– –	(+) –	– –
	51, 52, 58, 59	– –	+ +	+ +	+ +
	55	– –	– –	(+) +	– –

*: Antereo-posterior axes. +: Many zooids found. –: No hydroids. (+): Only a small number <20 of zooids attached.

body, where there are larger numbers of attached polyps, to prevent overcrowding.

Furthermore, hermaphrodites (see Celiberti *et al.*, 1998) were found in some specimens of *E. inquilina*, associated with a new host, *Chlamys glabra* (Table 2; *cf.* Piraino *et al.*, 1994) and also in another *Mytilus*-associated population from Lago Fusaro, near Naples, Italy, the type locality of this species.

No *Eugymnanthea* hydroids were found on the Adriatic coasts of Italy surveyed in the present study, Frigole and Torre del Serpe (*cf.* Kubota, 1989). Finally, no eutimid bivalve-inhabiting hydroids are associated with any bivalve population from Italy examined in the present study, differing from the case in Japanese waters (*cf.* Kubota, 2000), *i. e.* no findings of ancestral eirenids or eutimids whose hydroids harboured in bivalves.

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